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# BIOLOGICAL BULLETIN

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## INITIATION OF DEVELOPMENT IN NEREIS.

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If any apology be needed for merely adding to the long list of eggs susceptible to agents of artificial parthenogenesis it may be suggested that initiation of development in annelids possesses some interest since annelid ova respond only with difficulty to agents that induce development.<sup>1</sup> We need but recall the case of *Chaetopterus* (Loeb, '01b, Lillie '02, Allyn), of *Amphitrite* (Loeb, '01a, Scott), of *Podarke* (Treadwell), of the Pacific *Nereis* (Loeb, '13), of *Polynoe* (Loeb, '07, '08) and of *Nereis limbata* (Fischer, Lillie, '11, Loeb, '12b, '13)<sup>2</sup> to justify this statement. In all these eggs differentiation without cleavage is far easier to obtain than development closely simulating the normal. Among diverse agents few only will give cleavage in *Chaetopterus* (Allyn, Loeb and Wasteney). The case of *Thalassema* stands almost alone among annelids in giving development which is, according to Lefevre, to a surprising degree like the normal. Another instance among annelids of differentiation with cleavage artificially induced is worthy of note.

This report, however, on the initiation of development in *Nereis* by heat has, I think, special significance. The results here offered require an interpretation which concerns the fundamental theory of parthenogenesis and fertilization.

### EXPERIMENTAL.

Certain preliminary experiments date from 1913. The experiments given here were performed during June, July, and

<sup>1</sup> Bullot claims to have produced normal development in *Ophelia* with artificial means.

<sup>2</sup> Loeb's experiments with *Nereis* ('12) were apparently incomplete.

August 1914, at the Marine Biological Laboratory, Woods Hole. The majority of the experiments deal with the effect of heat on the *Nereis* egg. Under *A* these experiments are described. Under *B* are described experiments with KCl.

A. THE EFFECT OF WARMING ON THE INITIATION OF DEVELOPMENT IN NEREIS.

*Methods.*—At first all sea-water used was heated, usually not beyond  $75^{\circ}$  C., to destroy any spermatozoa possibly present, cooled, and vigorously shaken before the experiment. But this is unnecessary, as my observations showed. I have kept *Nereis* eggs in sea-water during the cool days of June for thirty-six hours without even jelly secretion. During several seasons I have never found eggs spontaneously developing in sea-water, although eggs occasionally extrude part of their jelly. Moreover, in not a single uninseminated control in ordinary sea-water was a developing egg ever found. In many experiments in addition to the uninseminated control a batch of eggs from the same animal as those warmed was inseminated. It was thus clearly proved that the eggs subjected to warming are in no wise abnormal. For fear of contamination, the needless inseminated control was discarded in the later work.

For a given experiment the following procedure was adopted:

A small flask or a large test tube with a measured quantity of sea-water was placed in a large beaker of sea-water. This was warmed over an alcohol flame and the temperature kept constant by the use of thermometers in the flask and in the beaker. The eggs were generally from one female; if from several small ones, they were mixed so that the inseminated or uninseminated controls and the warmed eggs were always the same. The eggs in the initial experiments (see below) were either from females cut in the warm sea-water or they were put in the warm sea-water dry; *i. e.*, from a thoroughly dried female which was pricked to cause the escape of eggs. Eggs were also subjected to heat after washing by changing the sea-water several times during various intervals of time. By means of a capillary pipette measured quantities of eggs were transferred after exposure at varying intervals to five or to one hundred c.c. of ordinary sea-water. The

experiments were performed during the morning and afternoon following the evening that the worms were captured. A few experiments were performed during the evening of capture.

The point to be emphasized is that washing in sea-water so modifies the eggs that they do not respond readily, or at all, to parthenogenetic treatment.

### THE EXPERIMENTS.

The experiments with heat may be divided into four groups as follows:

1. The initial experiments in which the eggs were cut from the animals while in the warm sea-water.
2. The experiments with dry eggs.
3. The experiments with eggs in warm "serum."
4. The experiments with washed eggs.

#### I. THE INITIAL EXPERIMENTS.

In the initial experiments, worms were cut in 5, 10, 25, 50, and 100 c.c. of sea-water at 30°, 32°, 33°, 34°, 35°, 36° C., the worms removed and the eggs exposed for from five to fifteen minutes.

The following experiments selected from a number give the details:

(a) July 22, 1:45 P.M. A female put in 100 c.c. of sea-water at 31° C. swims actively without discharging eggs. At 1:50, the temperature is 35° C. Eggs are cut out, the worm removed. Ten samples of eggs are removed to 5 c.c. of ordinary sea-water as follows: 2:03, 2:10, 2:25, 2:35, 2:45, 2:55, 3:05, 3:15, 3:25, 3:35 P.M.

At times the temperature rose to 36° C. and once to 36.5° C. Many eggs at the time of removal from the warm sea-water exhibited membranes standing off at an unusual distance, others were darker than normal, and a few had disintegrated. Later experiments showed that these changes are due to exposure at too high temperature. Even five minutes exposure at 37° C. will bring them about. The jelly is formed in the warm water, and often at 35° C. or above it is dissolved and disappears. This may be shown by examining eggs in India ink ground up in

sea-water. Many developing eggs are devoid of jelly hull, but the cortical changes are complete.

One hour and ten minutes after exposure, some eggs are in "blister" cleavages; that is, the protoplasm is irregularly budded. One hour and thirty minutes to two hours after the change to ordinary sea-water, among all gradations of cleavage-like patterns are some normal two and four-cell stages. The next day, Nos. 3 to 10 showed some real cleavages and a small per cent. of apparently normal swimming forms. Many are beaded or blistered, some are unsegmented "swimmers," and some two and four-cell swimming forms. Some eggs remain in the germinal vesicle stage.

(b) June 23, 11:00 A.M. A female placed in 50 c.c. of sea-water at 35.5° C. is rendered immobile but does not shed. Eggs are cut out at 11:02, the worm removed. Six samples of eggs are taken as follows: 11:18, 11:25, 11:34, 11:40, 11:45, 11:50.

Many eggs on removal from the warm sea-water show the jelly formed. The membranes after jelly formation are still a little farther from the eggs than in normal fertilization. Many eggs remain in the germinal vesicle stage with the cortex intact.

1:45 P.M. Fairly normal cleavages in Nos. 1 to 4.

June 24, 8:30 A.M. Swimming forms are found in the dishes. By far the best are those in Nos. 2 and 3.

The optimum time of exposure, therefore, lies between twenty-three and thirty-two minutes. Later experiments showed that the optimum exposure at 35° C. is at or near twenty-five minutes.

(c) June 24, several experiments were run at various temperatures. Those at 35° C. confirmed the findings of the previous ones. Temperatures ranging from 30° C. to 31° C. give no results; regardless of the length of exposure the eggs remain in the germinal vesicle stage.

The following experiment of June 24, at 33° C. is typical of a number of repetitions at this temperature:

(d) June 24, 11:15 A.M. A female placed in 25 c.c. of sea-water at 33° C. swims actively without discharging eggs. Eggs are cut out at 11:15 and samples taken at five-minute intervals up to 11:50. The temperature is practically constant. The samples taken are masses of eggs with the cortex wholly or (in

earlier ones) partially broken down. The cytoplasm is normal in color and the membranes normal.

1:35 P.M., many eggs are in cleavage.

4:00 P.M., many eggs are in late cleavage.

June 25, 9:00 A.M. The dishes show a good per cent. of very fine "swimmers." The cleavage seems almost normal.

Thirty-five minutes' exposure gives by far the highest percentage of swimming forms. As in all the experiments of this group, some eggs remain in the germinal vesicle stage with cortex intact.

If eggs be warmed in "egg-water" (sea-water charged by eggs that have remained in it for several hours) the results are no different.

To sum up, we find that eggs of *Nereis* cut out in warm sea-water and exposed to temperatures ranging from 33° to 35° C. develop with cleavage which is closely similar to the normal. Some eggs remain in the germinal vesicle stage. For the best percentage of swimming forms the optimum exposure at 35° is twenty-five minutes; at 33°, is thirty-five minutes.

## 2. EXPERIMENTS WITH DRY EGGS.

Many of the experiments with dry eggs were run with the washed egg series. In the majority of cases eggs from one female thoroughly dried on clean filter paper were received in a dry watch glass.<sup>1</sup> These eggs were divided into two lots; one lot warmed in sea-water at the given temperature and the other washed by changing the sea-water several times, allowed to settle, and after draining placed in the warm sea-water.

A large number of experiments was made with dry eggs, in the attempt to determine the quantitative relations early found to control the number of eggs developing. Thus, with smaller quantities of warm sea-water every single egg quickly forms jelly and at least ninety-eight per cent. cleave, but with larger quantities of warm sea-water the percentages are lower.

As Miss Allyn found for *Chaetopterus* cleavage appears to in-

<sup>1</sup> With dry eggs one must be careful for the mere drying will initiate changes as I have found. Eggs left on filter paper for from five to twenty minutes form jelly, a small per cent. cleave and a few swim.

terfere with the further development. I have never been able to get more than twenty per cent. of these eggs to reach the swimming stage. If one could determine definitely the quantitative relations this percentage might be increased. From the observations it appears that the optimum amount of warm water used varies; it depends upon the bulk of the eggs. While best results are got with small quantities of water, it is possible to use too little—three c.c. for instance, for the eggs for a large female. Jelly formation and cleavage are induced but swimming forms are less numerous than in the case of ten c.c. for about the same bulk of eggs.

The following are typical experiments of this group:

(a) July 16, 10:30 A.M. Eggs from a dry female in a dry watch glass are divided into two lots; one lot washed, the other transferred to 5 cc. of sea-water at 34° C. Samples out as follows: at 10:20, 10:50, and at five-minute intervals thereafter to 11:20.

2:00 P.M. Uninseminated control, no change. *Every single warmed egg had formed jelly: all have formed polar bodies.* At least half of these are in cleavage stages.

July 17. All dry eggs in some stage of cleavage, many of which are normal; some swimming forms in many of the dishes even after forty minutes' exposure.

(b) July 17, 9:58 A.M. Eggs from a dry female divided into two lots. Lot A in 5 c.c. of sea-water; Lot B in 20 c.c. of sea-water. Both exposed to 33° C. 10:01 jelly formation. Eight samples taken as follows: 10:05, 10:11, 10:16, 10:22, 10:27, 10:33, 10:38, and 10:43.

Lot A gave at least 95 per cent. of cleavage and a percentage of swimming forms in all dishes beginning with No. 3 (the 18-minute exposure). Lot B gave 75 per cent. of cleavage and best swimmers for 24, 29, and 35-minute exposures.

(c) Other experiments showed that the highest per cent. (100 per cent.) of jelly formation and of cleavage (98 to 99 per cent.) is in the smaller quantities of sea-water—5, 6, and 10 c.c.—whatever the temperature; a few swim. With larger quantities of sea-water at the various temperatures more eggs remain in the germinal vesicle stage. The lower exposures give most normal-looking swimming forms—trochophores scarcely to be

distinguished from the normal either while living or in sectioned material. The higher exposures give more abnormal swimming forms.

For comparisons I have selected the following tables from my notes to show the percentages of cleavage and of "swimmers" obtained with eggs from worms cut in warm sea-water and with dry eggs. It is apparent at once that while there is no appreciable difference in the percentages of swimming forms after warming either the "cut out" or the dry eggs, there is a marked difference in the percentages of cleaving eggs. This is the case in all the experiments.

July 12. Two females cut up at 9:55 A.M. in separate flasks of sea-water at 35° C. gave the following results:

*Female No. 1.*

	Sample Taken	Cleavage.	Swimming Forms.
No. 1	10:06	65%	1.5%
" 2	10:13	50	4
" 3	10:15	55	5
" 4	10:20	67	5
" 5	10:25	60	9
" 6	10:30	72	3
" 7	10:35	47	7
" 8	10:40	68	4—abnormal
" 9	10:45	62	very abnormal
" 10	10:50	33	very abnormal

*Female No. 2.*

	Sample Taken	Cleavage.	Swimming Forms.
No. 1	10:07	66%	3%
" 2	10:14	42	8
" 3	10:16	33	12
" 4	10:21	47	14
" 5	10:26	44	3
" 6	10:31	74	10
" 7	10:36	24	2—abnormal
" 8	10:41	81	5— "
" 9	10:46	32	very abnormal
" 10	10:51	17	very abnormal

July 20. Dry eggs in 30 cc. of warm water at 35° C. gave the following results:

Exposure.	Cleavage.	Swimming Forms.
18 minutes	90%	1%
23 "	"	15%
30 "	"	10%
35 "	"	7%



With dry eggs one may obtain 100 per cent. cleavage; with the eggs cut from worms in warm water one never gets more than 81 per cent. the average being very much lower as the figures given above show. With both kinds of eggs 20 per cent. swimming forms is the maximum, the optimum exposure for the various temperatures used being the same.

Experiments show that the use of warm "egg-water" does not improve the results.

### 3. THE EXPERIMENTS WITH SERUM EGGS.

The effect of warming *Nereis* eggs in the body fluids was studied with difficulty mainly because of the scarcity of body fluid in *Nereis*. As Lillie has pointed out this worm is little more than a bag of eggs. The amount of blood present is negligible and unavailable for warming experiments. I therefore adopted the method used by Lillie—that of cutting up spent females. In his study this juice gave results comparable to the perivisceral fluids in *Arbacia*. For an experiment I minced as many spent females as I could get, using a small quantity of sea-water; the juice thus obtained is designated as "serum." While I think that my experiments with this serum are conclusive I wish to point out that *Nereis* is not the most favorable form with which to establish the fact of serum inhibition—certainly this is true for the method I used. It may be stated at the outset that as Lillie found for both *Nereis* and *Arbacia* I have found repeatedly that the "serum" of *Nereis* quite definitely inhibits fertilization. Furthermore, just as definitely does the serum inhibit initiation of development with warming. I cite experiments to give the details:

(a) July 15, 10:55. Eggs from one fine large female previously dried are divided into four lots. Eleven spent females are finely minced to procure twenty drops of "serum." Ten drops of the "serum" is added to each of two dishes containing 3 c.c. of sea-water; eggs added to both. One lot is warmed at 34.5° C.—Lot A; Lot B inseminated. Samples of A are taken at five-minute intervals up to 11:35. 2:00 P.M., 1 per cent. of cleavage in both lots. Next day no swimming forms in either. Eggs from the same female, Lot C, warmed in sea-water and Lot D, inseminated, develop.

During the afternoon of July 15 this experiment was repeated with the same results.

(b) July 22. Eggs warmed at 34° C. in serum plus sea-water (serum from the bodies of seven spent females cut up in two c.c. of sea-water): 10 drops plus three c.c. sea-water, 10 drops plus five c.c. sea-water and 10 drops plus ten c.c. sea-water.

Eggs exposed for twenty-five minutes. Less than one per cent. developed in any dish.

During August these results with serum eggs were verified. The highest per cent. of swimming forms obtained was one per cent.; this was with a very dilute serum. Not only do the eggs fail to cleave but fail in the great number of cases even to form jelly. In some cases the development of eggs inseminated in serum was farther advanced than the serum warmed eggs. Since in the case of the initial experiments the worms were cut up in sea-water, it may be that failure of a percentage of eggs to cleave is due to the inhibition of the escaping blood and tissue juice. With the dry eggs cut quickly on the dry watch glass this escaping juice cannot so easily contaminate the eggs.

#### 4. THE EXPERIMENTS WITH WASHED EGGS.

In *Platynereis* sea-water definitely destroys the fertilizing power of the egg. Even minute quantities of sea-water will render the egg incapable of cleavage although the spermatozoa may penetrate. Moreover, if the eggs of one female remain in a small quantity of sea-water, 5 c.c., for instance, for *thirty seconds* their fertilizing power is lost. And yet in nature, inseminated eggs begin to be laid in many cases *five or six seconds* after copulation (see Just, '14). In *Nereis*, therefore, it was thought that washings in sea-water by frequent changes through several hours might act as the sea-water does in such a surprisingly short time on *Platynereis* eggs.

During the June *Nereis* run, then, as many experiments as possible were conducted to determine the "fertilizable" period by inseminating at intervals eggs that had remained in sea-water with and without frequent washings. Lillie has shown for *Arbacia* eggs that the capacity for being fertilized decreased with the decreased secretion of fertilizin. He finds for *Nereis* also very much the same relationship.

Without going into details, it may be said at the outset that the egg of *Nereis* gradually loses its power of being fertilized and eventually reaches the condition of the *Platynereis* egg where insemination induces maturation only. I cite a single experiment.

June 28, 9:10 P.M. Dishes of eggs Nos. 1, 2, and 3 were set aside. The next day at 2:10 P.M. each dish of eggs was drained and divided into two lots—*A* and *B*. Lot *A* in each case was inseminated in the water which had stood over the eggs for seventeen hours. Lot *B* of each dish was inseminated in fresh seawater. No eggs in either lot of No. 1 developed beyond maturation. In Lots *A* and *B* of Nos. 2 and 3, .1 per cent. or less went as far as the two-cell stage. Some eggs in all the dishes were in the germinal vesicle stage. No trochophores were found.

Eggs were frequently tied in bags of filter paper and placed in a beaker under running water for twelve hours. In other cases they were washed by changing the water at odd times during the day. It was found that eggs differ greatly with respect to the time that they must remain in sea-water before they lose their fertilizing power, but it may be clearly proved that washing or staling of *Nereis* eggs renders them incapable of being fertilized. This stage may be reached after three hours in seawater (cf. Just, '12).

This varying susceptibility proved very disappointing because I had suspected, not, of course, the degree of susceptibility present in *Platynereis*, but perhaps such as could be expressed more definitely.

Because of these results with washed and stale egg insemination, when the warm sea-water experiments were continued during the July *Nereis* "run" I was certainly unprepared for the results obtained. The following experiments are typical of a large number performed almost daily during the July and August "runs":

(a) July 11, 9:50 A.M. Eggs from one female divided into two lots; one lot put in sea-water. This lot transferred from the sea-water to warmed sea-water (35° C.). The eggs form jelly in the warm sea-water and make a mass which has to be shaken to obtain samples. 10:55, many have formed jelly and matured, but most retain jelly with germinal vesicle intact. Some of

these eggs again subjected to heat; no results. July 12. Very few, 1 in 1000, swimming.

(b) July 15, 3:20 P.M. Eggs cut out and washed, put in 6 c.c. of sea-water warmed at 35° C. Samples taken at five-minute intervals for forty minutes. Next day: Majority are in germinal vesicle stage, at least seventy-five per cent. Less than one per cent. swimming.

(c) July 16, 9:35 A.M. Eggs washed ten times evening before and five times during this morning. Two series: *A* inseminated, *B* in warmed sea-water at 3:50 samples taken (ten in all) at five-minute intervals. Uninseminated control.

July 17, 1:30 P.M. No development in uninseminated control (few have cytolized). Inseminated eggs show that few have formed jelly (ten to fifteen per cent.). One per cent. have cleaved and some of these swim. Of the warmed eggs at least ninety-five per cent. are in the germinal vesicle stage with cortex intact. Less than one per cent. have formed polar bodies.

(d) July 16, 10:30 A.M. Eggs from a dried female divided in two lots; one lot washed in 100 c.c. of sea-water by changing the water four times. 10:40 A.M. In warmed sea-water, 34° C. Samples out at five-minute intervals for sixty minutes.

2:00 P.M. At least ninety per cent. in the germinal vesicle stage, small per cent. form jelly and divide. Next day, none swim.

I was tempted to discredit my June experiments after the first of these findings. I could only convince myself after running series after series of washed and dry eggs along with eggs cut out directly into warmed sea-water. Most workers in inseminating eggs obtain the sexual products in separate dishes, and add sperm. Such procedure succeeds admirably with *Nereis* giving one hundred per cent. of cleavage. But if eggs be cut out of *Nereis* in sea-water, divided in two lots, and washed once or twice, one lot being inseminated and the other warmed we get the surprising result that while every single inseminated egg develops, few of the warmed go beyond maturation. If the water over the eggs be changed a few times in ten minutes, ninety per cent. warmed in sea-water fail even to mature.

This must mean that the egg of *Nereis* is so susceptible to

sea-water that warming fails after washing although fertilization is still possible. If fertilization be impossible (as in stale eggs) warming also produces no effect.

Washed or stale eggs warmed in sea-water charged by eggs that have remained in it for some time do not fare any better than those subjected to warmed sea-water; as in the first and second series of experiments this "egg water" makes no difference in the results.

I think that these facts are incontrovertible. Washing or even residence in sea-water for a short time interferes seriously with the effect of heat in initiating development.

Study of insemination of dry and washed eggs was made. Apparently there is a difference here of response to the spermatozoon. The dry egg is more irritable, jelly formation being extremely rapid. This is true of dry eggs inseminated in small quantities of sea-water. This behavior recalls that of *Platynereis*.

These results, moreover, might suggest that our methods are much too crude in the study of these extremely sensitive cells—the egg and the spermatozoon.

Summing up we may say concerning the effects of warming on the eggs of *Nereis*: (1) *That while eggs cut out of worms in the warm sea-water form jelly and divide in large numbers, a small per cent. swimming, some remain in the germinal vesicle stage.* (2) *That at least ninety-eight per cent. of the dry eggs form jelly almost all of which cleave: twenty per cent. become trochophores closely resembling the normal.* (3) *That eggs in "serum" fail to develop except in very small numbers.* (4) *That washed eggs even after but two or three washings develop if at all in small numbers.*

#### B. EFFECT OF KCl IN THE INITIATION OF DEVELOPMENT.

According to Fischer the eggs of *Nereis* after treatment with KCl will go through cleavage and produce trochophores. Lillie ('11), however, could not get the eggs after KCl treatment to go beyond maturation. During three seasons this had been my experience. This summer I studied the effect of KCl on washed and unwashed eggs.

If the eggs be washed two or three times before exposing to

the action of KCl every egg matures but never more than one in a thousand swims. If the eggs be allowed to remain in sea-water from two to twelve hours with frequent changes of sea water the results are about the same. Dry eggs subjected to KCl treatment mature, cleave once or twice, and produce, in one experiment at least, seven per cent. of swimming forms made up of unsegmented two and four-cell "swimmers."

#### THE EXPERIMENTS.

5, 10, 15, 20 and 25 per cent. 2.5M KCl were used. It was found that 15 per cent. 2.5M KCl in sea-water gave the best results. Typical experiments follow:

(a) August 12, 10:54 A.M. Lot *A*: Eggs from two females cut in 80 c.c. of 20 per cent. 2.5M KCl at 10:54. Lot *B*: Eggs from one dry female put in 3 c.c. of 20 per cent. 2.5M KCl. At 11:00 jelly formation in both. Samples of eggs taken from *A* and *B* as follows:

No. 1.....	11:00
No. 2.....	11:10
No. 3.....	11:17
No. 4.....	11:25
No. 5.....	11:33
No. 6.....	11:40
No. 7.....	11:50
No. 8.....	12:00

August 13, 12:00 M. Dry eggs of August 12 (Lots *A* and *B*). All matured; some cleavage-like processes and some swimming forms after twenty minutes' exposure or more. Highest percentage (five) of swimmers after fifty minutes' exposure. These are unsegmented, two and four-cell swimmers.

(b) August 12, 12:05 P.M. Water changed three times on eggs during three hours and then placed in 20 c.c. of 20 per cent. 2.5 M KCl in sea-water. Samples taken at five-minute intervals up to 1:00 P.M.

August 13, 1:00 P.M. Washed eggs of August 12, all matured; 1 in 1,000 swim.

Experiments during August 13 and 14 with fifteen per cent. 2.5 M KCl gave about the same results.

(c) August 15, 9:30 A.M. Two females quickly cut in 10 c.c.

of 15 per cent. 2.5 M KCl and removed. Samples taken as follows:

No. 1.....	10:35
" 2.....	10:50
" 3.....	11:05
" 4.....	11:20
" 5.....	11:40
" 6.....	12:00
" 7.....	1:25

These gave the next day swimming forms as follows:

No. 1.....	4 per cent
" 2.....	7 " "
" 3.....	4 " "
" 4.....	2 " "
" 5.....	2 " "
" 6.....	2 " "
" 7.....	3 " "

(d) On August 17, eggs were washed by changing the water six times in five hours then subjected to 15 per cent. 2.5 M KCl in sea-water, samples being taken at sixty minutes and thereafter at five-minute intervals. One tenth per cent. (.1%) was the best result after sixty minutes in the KCl sea-water.

(e) *Combination of KCl with Heat.*<sup>1</sup>—An experiment of last summer was repeated except that dry eggs were used and the minimum exposure, five minutes, of the series the only one tried. The protocol follows:

August 17, 1:25 P.M. Dry eggs are put in 10 c.c. of 15 per cent. 2.5 M KCl in sea-water for five minutes; jelly formation almost at once. Eggs are then placed in 50 c.c. of sea-water at 35° C. Four lots removed to 100 c.c. of sea-water as follows:

No. 1.....	1:55
" 2.....	2:00
" 3.....	2:05
" 4.....	2:10

The next day, at 10:30 A.M., the percentages of swimming forms, largely unsegmented found were as follows:

No. 1.....	(25 minutes' exposure to heat).....	3 per cent.
" 2.....	(30 " " " " " ).....	7 " "
" 3.....	(35 " " " " " ).....	27 " "
" 4.....	(40 " " " " " ).....	25 " "

<sup>1</sup> It will be recalled that Allyn used a combination treatment of KCl and heat on the egg of *Chaetopterus* with rather different results from those mentioned here with *Nereis*. Her method however, was different.

It appears, therefore, that with KCl, and KCl and heat, washed and unwashed eggs alike will maturate, but that the dry eggs alone respond with cleavage or the production of swimmers.

#### DISCUSSION.

In the egg of *Nereis* Lillie discovered a substance, *fertilizin*, which has the property of agglutinating *Nereis* sperm. This substance may be detected in the water in which the eggs have remained for a short time. If, however, the eggs be washed by changing the water two or three times the fertilizin is no longer secreted in detectable quantities, *i. e.*, there is not enough to agglutinate the sperm. Such eggs are none the less fertilizable by sperm, giving off at the time of insemination more fertilizin, all of which is then utilized or completely thrown off during the cortical changes. It therefore follows that at the time of shedding the egg is laden with free fertilizin ready for secretion. This conclusion is supported by additional facts. In the first place I have pointed out above that the dry egg or egg in small quantities of sea-water is hyper-irritable—that is, if jelly formation may be taken as index. If one inseminates the eggs of *Nereis* dry or in small quantities of sea-water the jelly formation is extremely rapid. Jelly formation is correspondingly slow in washed and stale eggs. The breeding behavior noted night after night for several seasons is significant: freshly shed eggs at the surface of the sea excite numbers of males to shed their sperm around the shedding or recently spent female. Lillie's experiments (Lillie and Just) on this sperm shedding reflex, moreover, prove that the egg loses fertilizin once in the sea-water. The "dry" and "washed" eggs of my experiments, then, are physiologically different: the dry egg has all its available fertilizin content, the washed egg has secreted part of this substance.

Lillie has shown that the eggs of *Nereis* will not fertilize in the tissue juices of the animal; my experiments show also that the body juice of spent females inhibits fertilization. Unlike the washed egg, the "serum" eggs possess fertilizin but its action is inhibited.

But it is on the basis of experiments on *Arbacia* that Lillie has developed the fertilizin theory as an explanation of the me-



chanism of fertilization. Without going into details it may be said that in *Arbacia* it is found that the egg secretes a substance, fertilizin, whose presence is capable of quantitative determination and which is necessary for fertilization since first, eggs washed free of it are no longer capable for fertilization; second, fertilized eggs no longer secrete it; and third, eggs after membrane formation with butyric acid are not capable of fertilization and do not give off the substance. The perivisceral fluid of *Arbacia*, moreover, produces an inhibiting effect on fertilization preventing the action of fertilizin on the egg.

My results with warming *Nereis* eggs parallel to a striking degree these facts brought out in the studies of fertilization in *Nereis* and *Arbacia* (Lillie, '12, '13a, '13b, '14). Eggs washed free of the bulk of fertilizin will not develop however long the warming treatment lasts; serum inhibits the artificial initiation of developmental processes; only the dry eggs with their full content of fertilizin when suddenly shocked with elevation of temperature respond with jelly formation and cleavage. It would seem, therefore, as I have suggested for *Platynereis*, that fertilizin is just as essential for artificial initiation as for normal fertilization. The difference seems to be that for artificial initiation more fertilizin is required. Further attempts at Woods Hole this summer to induce artificial parthenogenesis in *Platynereis* strengthen this belief; a percentage of *Platynereis* eggs will fertilize in small quantities of sea-water; the same bulk of eggs in the same amount of water fail to respond when subjected to warming.

If, therefore, as Loeb ('12a) says, "fertilization is primarily and essentially artificial parthenogenesis"; or if "a theory of fertilization must also be a theory of parthenogenesis at least for the phenomena common to both"; and if "similarly a theory of fertilization must be consistent with the facts of parthenogenesis" as Lillie ('14) suggests; these experiments, we are forced to conclude, make another link in the chain of evidence which supports the theory that fertilization is essentially a process of the egg. The spermatozoon initiates the development of the egg, as does warming, through the activation and the binding of the fertilizin.

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